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MANAMAKO NO SHUBYOSEISAN TO CHINAMAKO NO SHIIKUHOHO NO KENTO* p. 37

(ON THE ARTIFICIAL SEEDLING AND REARING EXPERIMENTS OF SETTLED JUVENILE OF THE SEA CUCUMBER Stichopus japonicus)

Zenpei IKEDA and Katsusuke KATAYAMA

Showa 58nendo Okayama Suishi Jiho (Bull. Okayama Fish. Exp. Stn., 1984)

The study of the production of artificial seedlings and the intermediate rearing methods was continued from the previous year[1)] for the purpose of mass production of artificial seedlings and release of sea cucumbers Stichopus japonicus. Results are reported below.

Materials and methods

Production of artificial seedlings

Aonamako (green sea cucumbers) which were caught off the coast of Ushimado from 21st to 26th of February in 1983 were used as parent sea cucumbers. These sea cucumbers were put in an indoor concrete tank. They were fed once a week with compound feed for rearing sea cucumbers sold on the market and were reared in running water for about a month and a half. One week before the collection of eggs, they

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were moved to a small tank and were kept without feed.

The same methods reported in the previous bulletin[2)] were used for the induction of egg-deposition, the collection of eggs and the rearing of larvae. 26 individuals whose total weight was 440-1,125 g (mean total weight 710 g) were used as the parent sea cucumbers. 1 kL FRP black tanks (water depth 60 cm, hereinafter called the 1 kL tank) were used for the rearing tank. For feed, the same amounts of Pavlova lutheri and Chaetoceros sp. were used as in the previous year. These were added every day so that the concentration was 1.0-1.5 x 10^4 cells per millilitre of rearing water after calculating the remaining feed until the early period of juveniles and 1.5 x 10^4 cells after that period. In the latter period of Auricularia, an attachment apparatus made of scallop shells was hung down in 3 tanks out of 4.

Rearing of settled juveniles of sea cucumber

Two methods given in Figs. 1 and 2 were used for rearing. The method given in Fig. 1 was to set in water about 10 cm from the bottom of the 1 kL tank a polyethylene container (hereinafter called the settling tank) which was 39 x 32 x 23 (H) cm and in which a plankton net was spread along the bottom. Then juveniles of body length 0.3 mm were put in the settling tank and reared. The number of juveniles kept per tank was 529-1,300. The tank was covered with a black vinyl sheet so that the inside the tank was almost pitch dark.

p. 38

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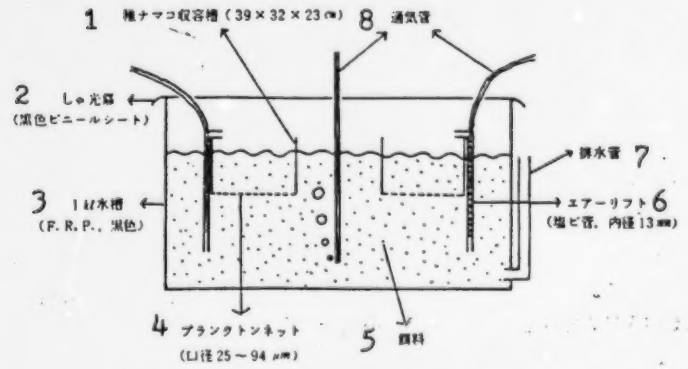

第1図 稚ナマコの飼育水槽－1

Fig. 1 Rearing tank of settled juvenile sea cucumbers---I

1. Settling tank for juvenile sea cucumbers, 2. Shading screen (black vinyl sheet), 3. 1 kL tank (F.R.P., black), 4. Plankton net (mesh 25-94 μm), 5. Feed, 6. Air lift (vinyl chloride pipe, inner diameter 13 mm), 7. Drainpipe, 8. Ventilation pipe

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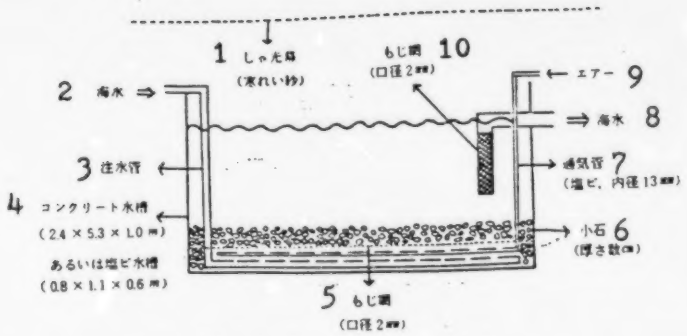
The feed, P. lutheri and C. sp., were given in the same amounts. The feed was put in the tank so as to enter the settling tank with sea water by means of each air lift. The pumping capacity of the air lift was about 300 L per day for the first 40 days of rearing and was increased to about 600 L after that.

For the rearing water, about 1 kL of water was changed taking 4 to 6 hours once every 2 or 3 days by overflowing through the drainpipe from an opening near the bottom. When the water level in the settling tank increased due to clogging of the net, water was poured in for washing.

The method given in Fig. 2 was to keep the settled juvenile sea cucumbers in the tank in which pebbles were



第2図 椎ナマコの飼育水槽－Ⅱ

Fig. 2 Rearing tank of settled juvenile sea cucumbers---II

1. Shading screen (cheese cloth), 2. Sea water, 3. Water pipe, 4. Concrete tank or vinyl chloride tank, 5. Japanese minnow netting (mesh: 2 mm), 6. Pebbles (thickness several cm), 7. Ventilation pipe (vinyl chloride, inner diameter 13 mm), 8. Sea water, 9. Air, 10. Japanese minnow netting (mesh : 2mm)

spread at the bottom. They were reared using diatoms which attach to the pebbles for feed. Two tanks were used. One was 2.4 x 5.3 m with a water level of 0.7 m (hereinafter called the 9 kL tank) and the other 0.8 x 1.1 m with a water level of 0.45 m (hereinafter called the 0.4 kL tank). 31,000 and 1,500 settled juvenile sea cucumbers respectively were kept in each tank.

Running water was used 1 week before the beginning of rearing and diatoms attached to the pebbles were propagated in advance. The upper part of the tank was covered with a cheese cloth of a percentage of shading of 50 % for the first 19 days of rearing and after that period, the shading cloth was doubled so that illumination should be about 5 Klux at noon on a clear day. The size of the pebble for the 9 kL

tank was 5-12 mm and 1-3 cm for the 0.4 kL tank.

Copepoda extermination test

A 2 L beaker was used for the test. Copepoda and settled juvenile sea cucumbers were kept for a fixed time in sea water to which 0.1-5.0 mg of DEP (mazoten for marine products) were added per litre of sea water. The number of survivals was counted after 3, 24 and 72 hours respectively.

In principle, the mean body lengths of larvae and settled juvenile sea cucumbers were determined by measuring 30 individuals after fixing in formalin. In the case of settled juvenile sea cucumbers, as they grew the difference in body lengths between live individuals and fixed specimen became great. Therefore the live body lengths are also given.

Results and discussion

Production of artificial seedlings

The parent sea cucumbers had taken a fair amount of diatoms attached to the tank walls and the compound feed during the rearing period until early April. However, although measurements were not taken, the body size became clearly smaller by the time of the collection of eggs. It is thought to be necessary to study in future the method of management of rearing including the quality and quantity of feed.

On 13 April, the parent sea cucumbers were transferred from the tank with rearing temperature to one with temperature 5°C higher and heated and stimulated for the induction of

Table 1 Rearing results of sea cucumber larvae

第1表 ナマコ幼生飼育結果

A 水槽 No.	B 供試幼生 個体数 a (×10⁴個)	平均体長 b (mm)	C 取上げ個体 個体数 a (×10⁴個)	歩留り b (%)	平均体長 c (mm)	飼育期間 D (日)	飼育水温※ E (℃)
1	100.2	0.3	37.9	37.8	0.3	26	16.6～22.9
2	99.1	〃	40.7	41.1	0.3	27	16.9～22.7
3	99.4	〃	24.4	24.5	0.2	27	16.6～22.6
4	99.4	〃	0.6	0.6	3.4※※	90	16.3～27.5

※ 15時頃測定。
※※ 生標本の体長。

A. Tank, B. Sample larvae: a. Number of individuals, b. Mean body length, C. Harvested individuals: a. Number of individuals, b. Yield, c. Mean body length, D. Rearing period (day), E. Rearing water temperature
* Measured at about 15 hours.
** Body length of live specimen.

egg-deposition. After 50 minutes, males began to react and after 60 minutes females. After 2 hours 30 minutes, 5 males had emitted sperm and 5 females spawned. The induction rate was 38.5 %. As in previous year, there were many sunken eggs.

The rearing results of larvae are given in Table 1. The rearing process commenced on 15 April with transfer of 100 x 10^4 of Auricularia per tank. After 12-13 days, they metamorphosed to Doriolaria. After 19-21 days, most of them sank and almost no floating individuals were recognized in any of the 4 tanks. After 26-27 days, 24.4-40.7 x 10^4 per tank of settled juvenile sea cucumbers of body length 0.2-0.3 mm were harvested from Tanks Nos. 1, 2 and 3. The

Table 2 Attachment condition of settled juvenile sea cucumbers (unit x 10^4) p. 39

第2表 稚ナマコの付着状況（単位×10^4）

A 水槽No	B 付着数		
	a 全数	b付着器(%)	c水槽壁(%)
1	37.9	20.8 (55)	17.1
2	40.7	28.1 (69)	12.6
3	24.4	–	24.4
4	0.6	0.5 (83)	0.1

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A. Tank, B. Number attached: a. Total number, b. Attachment apparatus, c. Tank wall

yield was 24.5-41.1 %.

Rearing was continued for Tank No. 4 after the harvest from Tanks No. 1-3. After 90 days, 6,000 individuals of mean body length 3.4 mm (live body length) were harvested. The yield was 0.6 % and extremely low compared with other tanks. The number of settled juvenile sea cucumbers on the attachment apparatus after 27 days of rearing was the same as that for Tanks No. 1 and 2. Therefore it is thought that the number decreased after that time. One of the factors in the decrease is the increase of Copepoda from 1 month after the commencement of rearing. At the Buzen Fisheries Experiment Station[3] in Fukuoka Prefecture, settled juvenile sea cucumbers of body lengths 0.3 and 1.0 mm were observed to be eaten by Tigriopus, a species of Copepoda. The name of the species of Copepoda which increased at this time is not known. However, their feeding upon the settled juvenile sea cucumbers can be given as one of the factors in the decrease.

Furthermore the rearing water sometimes became muddy and white. When this occurred, water was changed every day. It was thought that muddy rearing water and the loss of settled juvenile sea cucumbers which were washed away are also factors in the decrease.

The numbers of settled juvenile sea cucumbers which attached to the attachment apparatus which were put in Tanks No. 1, 2 and 4 are given in Table 2. The rate of attachment to the attachment apparatus was comparatively high viz. 55 and 69 % for the rearing period of 26 and 27 days. The number of settled juvenile sea cucumbers was higher in Tanks No. 1 and 2 than that in Tank No. 3 in which no attachment apparatus was hung. The difference was thought to be due to the presence of the attachment apparatus. The results from the year before the previous year, in which some tanks did not have the attachment apparatus, showed the same tendency.

Rearing of settled juvenile sea cucumbers---I

Results are given in Table 3. On 12 May, juvenile sea cucumbers were put in the settling tank shown in Fig. 1 and the rearing commenced. The body length, the number of individuals harvested and the yield after that date are as follows: in Tank No. 1 after 19 days, 0.[illegible] mm and 45.1 %, in Tank No. 2 after 40 days, 1.4 mm (live body length 2.1 mm), 151 individuals and 28.5 %, in Tank No. 3 after 75 days, 3.0 mm (live body length 6.1 mm), 62 individuals and 9.5 %. The rearing density differed according to the settling tank.

Table 3 Rearing results of settled juvenile sea cucumbers---I

第3表 種ナマコの飼育結果－I

A 収容槽 No.	B 供試個体		C 取上げ個体			飼育日数
	個体数 a (個)	平均体長 b (mm)	個体数 a (個)	歩留り b (%)	平均体長 c (mm)	D (日)
1	1,300	0.29	586	45.1	0.6	19
2	529	〃	151	28.5	1.4 (2.1)※	40
3	651	〃	62	9.5	3.0 (6.1)※	75

※（ ）内は生標本の体長

A. Settling tank, B. Sample individual: a. Number of individuals, b. Mean body length, C. Harvested individual: a. Number of individuals, b. Yield, c. Mean body length, D. Number of rearing days
* Numbers in the parentheses indicate the body length of live specimen.

However, many settled juvenile sea cucumbers died at an early stage of rearing and a tendency was observed that they continued to die off gradually.

The growth was comparatively good until 1 month of rearing. However, after that time, almost no growth was observed even with an increase in the amount of feed as given in Table 4. After 35 days of rearing, as Copepoda increased, p. 40 the feed on the net at the bottom was seldom seen. From this fact, it was thought that lack of growth was caused by a lack of feed due to competition for the feed with Copepoda. On the basis of the test results which are explained in a later chapter of this paper, after 56 days, DEP was added to a concentration of 1.0 mg per litre of sea water and bathed for 8 hours in order to exterminate Copepoda. After bathing, running water was used until the following

Table 4 Feed concentration (unit 10^4 cells/1 mL rearing water)

第1表 餌料濃度（単位 10^4 cells／飼育水 1 ml）

A 餌料 ＼ B 期間	a 飼育開始～20日後	b 21日～40日後	c 41日～53日後	d 54日～75日後
P. lutheri *Chaetoceros sp*	0.2	0.4	0.6～0.8	1.0

A. Feed, B. Period: a. Commencement of rearing-20 days, b. 21-40 days, c. 41-53 days, d. 54-75 days

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morning. 4-5 days after the treatment, growth was observed again. However, after about 70 days, just before the harvest, an increase of copepoda was observed again.

The mesh size of the net spread at the bottom was 25 μm at the onset of the rearing. However, it was clogged quickly due to feed and excrement. Therefore the net was replaced with one of larger mesh size 42 μm after 3 days, 74 μm after 10 days and 94 μm after 21 days.

In this rearing experiment, many individuals died at the beginning and the number was reduced to half after 19 days. A tendency was observed that deaths continued gradually after that time. After 75 days, on the date of harvest, the body length was 3.0 mm and the yield was 9.5 %. The delay in growth was observed due to a lack of feed caused by competition for feed with copepoda which increased during the rearing period. It is thought that deaths were caused by lack of feed and by falling prey to copepoda as explained previously. Therefore it is necessary to exterminate copepoda at an early stage. Furthermore it is necessary to study the rearing density and

Table 5 Results of rearing of settled juvenile sea cucumbers---II

第5表 稚ナマコの飼育結果－Ⅱ

A 水槽の大きさ (kℓ)	B 供試個体		C 取上げ個体				飼育水温※※ D
	個体数 a	平均体長 b (㎜)	個体数 a	歩留り b (%)	平均体長(㎜)※ c 体長範囲	平均体重 d (g)	
9	31,000	0.3	114	0.4	16.5 7～29	0.16	16.3～29.7
0.4	1,500	〃	46	3.1	30.8 14～61	0.86	16.8～29.6

※ 生標本の体長
※※ 15時頃測定

A. Size of tank, B. Sample individual: a. Number of individuals, b. Mean body length, C. Harvested individual: a. Number of individuals, b. Yield, c. Mean body length*, Range of body length, d. Mean body weight, D. Rearing water temperature**
* Body length of live specimen
** Measured at about 15 hours

the amount of feed given. If the results of the study shows an improvement in the growth rate and the yield so that high density rearing may be possible, this rearing method is thought to be a suitable rearing method for settled juvenile sea cucumbers.

Another rearing method of settled juvenile sea cucumbers was tried by using the tank shown in Fig. 1 and by giving commercial compound feed for sea cucumber seedlings and compound feed for water-fleas Daphnia pulex. However, most individuals died within a month of rearing.

Rearing of settled juvenile sea cucumbers---II

Results of rearing are given in Table 5. On 11 May, juvenile sea cucumbers were put in the 9 and 0.4 kL tanks and the rearing commenced.

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The attached diatoms increased three times during the rearing period but they decreased considerably in between those three times. The mesh size of the net for the prevention of juvenile sea cucumbers being washed away was 0.3 mm at the beginning of the rearing. However, since it was clogged quickly, it was replaced by a net of 2 mm mesh size one week after the rearing commenced.

After 126 days of rearing, 114 settled juvenile sea cucumbers of body length 16.5 mm (live body length) were harvested from the 9 kL tank and 46 individuals of body length 30.8 mm (live body length) from the 0.4 kL tank. The yields were 0.4 and 3.1 % and as low as that of the previous year which was 1.5 %.

The factors in the decrease are thought to be the extreme increase and decrease of attached diatoms and the loss from washing away of settled juvenile sea cucumbers as in the previous year. However, this was not proven.

It is thought that natural juvenile sea cucumbers feed on attached diatoms. However, the results, including the results from this study, of rearing by giving attached diatoms in tanks on land indicated a production of a mere several hundred juvenile sea cucumbers of body length 1-3 cm[4-9]. However, at the Buzen Fisheries Experiment Station[3] in Fukuoka Prefecture, 2.6×10^4 settled juvenile sea cucumbers of body length 1-2 cm have been produced by using 6 1 kL tanks which contained a wavy plate to which diatoms were

Table 6 Results of copepoda extermination test p. 41

第6表 コペポーダ駆除試験結果

A DEP剤濃度 ppm/l	B 生存個体数 a コペポーダ / b 稚ナマコ（生存率%） t：開始時 a	3時間後 b	24時間後 c	72時間後 d
0	++	++	++	++
	32			29 (90.6)
0.1	++	++	+	+
	17			13 (76.5)
0.2	++	+	+	−
	22			16 (72.7)
0.5	++	+	−	−
	26			23 (88.5)
1.0	++	+	−	−
	18			15 (88.9)
2.0	++	−	−	−
	26			20 (76.9)
5.0	++	−	−	−
	16			14 (87.5)

コペポーダの場合は＋＋が多い，＋が少い，－がへい死（横あるいはあお向けになった状態。）。
水温は23.6～24.5℃（15時頃測定）

A. DEP concentration, B. Number of surviving individuals: a. copepoda, b. settled juvenile sea cucumbers (survival rate), t: a. At the onset, b. After 3 hours, c. After 24 hours, d. After 72 hours
In the case of copepoda: ++ denotes many, + denotes a few, - denotes death (lying sideways or on their backs).
Water temperature was 23.6-24.5°C (Measured at about 15 hours).

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attached under a low illumination of several hundred lux with the highest yield being 19.4 %. In this case, it was pointed out that one of the factors for the decrease was that the juveniles were eaten by copepoda. This indicated that there are problems in the rearing methods other than those of feed. It is thought that an improvement in the yield can be achieved by bettering the rearing method.

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Copepoda extermination test

Test results are given in Table 6. The test was conducted by putting about 300 copepoda and settled juvenile sea cucumbers in a beaker containing 2 L of sea water with prescribed DEP concentrations. Bathing time for 0.1-1.0 mg was 24 hours, for 2.0 and 5.0 mg, 3 hours. After bathing the sea water was changed completely. All copepoda died after 72 hours at 0.2 mg, after 24 hours at 0.5 and 1.0 mg and after 3 hours at 2.0 and 5.0 mg. However, they were alive even after 72 hours at below 0.1 mg. Settled juvenile sea cucumbers were alive after 72 hours regardless of DEP concentrations. The yield was 72.7-90.6 % and a little low. One of the factors for the low yield was thought to be deaths due to handling.

From the above results, it is thought that copepoda alone can be exterminated without affecting settled juvenile sea cucumbers of body length 1.9 mm and 2 months after hatching if DEP is added to sea water so that the concentration becomes 0.2-5.0 mg per litre.

The Buzen Experiment Station[3)] in Fukuoka Prefecture conducted a study of extermination methods of Tigriopus using DDP and DEP emulsion. The results showed that both chemicals can exterminate the adults without affecting settled juvenile sea cucumbers of body length 0.3-2.0 mm at a concentration of 0.1-1.0 mg per litre of sea water, however, when the eggs are returned to normal sea water, they hatch

and become normal Nauplius. As described before, copepoda were exterminated using DEP during the rearing of settled juvenile sea cucumbers, however, copepoda increased after 2 weeks. Therefore it is thought that copepoda extermination using DEP should be repeated periodically.

Summary

1. For the purpose of mass production of artificial seedlings of sea cucumbers for release, methods of production of artificial seedlings and of intermediate rearing of sea cucumbers were discussed.

2. The spawning was carried out within 2.5 hours from the commencement of thermal stimulation. The induction ratio was 38.5 %.

3. Settled juvenile sea cucumbers were reared in the same manner as in the previous year using 4 1 kL tanks. After 26-27 days, 24.4-40.7 x 10^4 settled sea cucumbers of body length about 0.3 mm were harvested. The yield was 24.5-41.1 %.

4. The rearing was continued after that time. The number of settled juvenile sea cucumbers of body lenth 3.4 mm harvested after 90 days was 6,000. The yield was 0.6 % which was very low.

5. The attachment ratio of settled juvenile sea cucumbers for the attachment apparatus was 55-69 %. This was a comparatively high rate indicating the effectiveness of the apparatus.

6. Two methods were used for rearing settled juvenile sea cucumbers of body length 0.3 mm. In one method, a settling tank for juvenile sea cucumbers in which a plankton net was spread at the bottom was set afloat in a 1 kL tank. Pavlova lutheri and Chaetoceros sp. were used for the feed. After 75 days, 62 settled juvenile sea cucumbers of body length 3.0 mm (live body length 6.1 mm) were produced. The yield was 9.5 %.

7. In this method, copepoda extermination was carried out and the future rearing density and the amount of feed were studied. As a result, the growth rate and the yield were improved. If high density rearing were to become possible, this method is thought to be a promising one for rearing of settled juvenile sea cucumbers. p. 42

8. Using the same rearing tank, settled juvenile sea cucumbers were reared using compound feed for seedlings of sea cucumbers and that for water-fleas sold on the market. In each case, almost all settled juvenile sea cucumbers died within a month.

9. In other method, 2 tanks, one large and the other small, were used in which pebbles were spread at the bottom and diatoms which were attached to the pebbles were used for the feed. After 126 days, 114 settled juvenile sea cucumbers of body length 16.5 mm and 46 of body length 30.8 mm were produced. The yields were low, 0.3 and 3.1 % respectively.

10. However, the Buzen Fisheries Experiment Station,

Fukuoka Prefecture reared settled juvenile sea cucumbers under a low illumination of several hundred lux. 2.6×10^4 settled juvenile sea cucumbers of body length 1-2 cm were produced and the highest yield was 19.4 %. Furthermore loss of settled juvenile sea cucumbers to copepoda has been pointed out as one of the factors in the decrease during the rearing. This suggests that if the method of rearing is improved, better yields can be obtained even if the attached diatoms are used for feed.

11. It was found that copepoda which increased during the rearing of settled juvenile sea cucumbers can be exterminated using DEP. It was also thought that it is necessary to carry out copepoda extermination periodically.

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Manamako no Shubyo Seisan ni tsuite

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Manamako no Shubyo Seisan ni tsuite II

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